Managing resistance to the insect growth regulator, pyriproxyfen, in *Bemisia tabaci*†‡

A Rami Horowitz,[1]* Zmira Mendelson,[1] Matthew Cahill,[2]§ Ian Denholm[2] and Isaac Ishaaya[1]

[1] *Department of Entomology, Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel*
[2] *Department of Biological and Ecological Chemistry, IACR-Rothamsted, Harpenden, Hertfordshire AL5 2JQ, UK*

Abstract: The insect growth regulator pyriproxyfen (a juvenoid) effectively inhibits the hatching of eggs of the tobacco or cotton whitefly, *Bemisia tabaci*, as well as causing pupal mortality. Since 1991, this insecticide has been one of the main agents for controlling *B tabaci* on Israeli cotton. Seasonal trends of susceptibility to pyriproxyfen in field populations were monitored from June (prior to treatment) through late summer at different locations in Israel. After seven years of pyriproxyfen use within an insecticide resistance management strategy that limits this insecticide to a single application per season, susceptibility has been maintained in many areas. In other locations where pyriproxyfen had been used against geographically isolated populations of *B tabaci*, moderate to high levels of resistance have been observed. Ecological and agronomic factors that may contribute to geographical variation in selection for resistance are discussed.

The dynamics of pyriproxyfen-susceptible and -resistant populations of *B tabaci* following a single application of pyriproxyfen were investigated under simulated field conditions in the laboratory. The susceptible population was suppressed very effectively, whereas effects of pyriproxyfen against the resistant population were much more transient. Differences in the productivity of susceptible and resistant strains in the absence of pyriproxyfen treatment could reflect a fitness cost accounting for observed reductions in resistance levels between seasons in the field. They may also explain why, following a recent reduction in the use of pyriproxyfen in cotton fields, resistance in 1998 declined to levels observed in 1995/6.

Keywords: pyriproxyfen; *Bemisia tabaci;* IGR; insecticide resistance; resistance management; Israel; cotton

1 INTRODUCTION

The insect growth regulator (IGR) pyriproxyfen exhibits juvenoidal activity and efficiently inhibits hatch of *Bemisia tabaci* (Gennadius) eggs, directly or transovarially. Pyriproxyfen also affects nymphs by suppressing adult emergence, resulting in pupal mortality.[1,2]

In the cotton pest management programmes in Israel and in Arizona, this insecticide has become one of the main agents for controlling *B tabaci*.[3–5] Pyriproxyfen was allocated for use in the third stage of the Israeli cotton strategy and was restricted to one treatment per season, applied between mid-July and mid-August. Thereafter, growers were recommended to use another IGR, the chitin inhibitor buprofezin, and various adulticides, if needed. Monitoring for resistance to pyriproxyfen has been conducted since its introduction to Israeli cotton in 1991. Seasonal trends of susceptibility to pyriproxyfen in *B tabaci* field populations were monitored from June (prior to treatment) through late summer, when, initially, only a slight increase in tolerance was observed. Due to the restricted use of novel compounds in cotton fields, and consequently a

* Correspondence to: A Rami Horowitz, Department of Entomology, Agricultural Research Organisation, The Volcani Center, Bet Dagan 50250, Israel
E-mail: hrami@netvision.net.il
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reduction of selection pressure, these insecticides could be re-applied in the following season when the pest populations had retained susceptibility to the compound.

This is in contrast to the situation in a rose greenhouse where, after three successive applications, high (greater than 500-fold) resistance to pyriproxyfen (when measured in terms of egg hatch) was demonstrated in whiteflies.[6] Despite the proven potential of *B tabaci* to develop high resistance to this chemical in enclosed environments, the response of whiteflies on cotton to pyriproxyfen has mostly remained stable. In 1995, however, there were localized outbreaks of pyriproxyfen resistance in some cotton fields.[5]

In this paper, data on the detection and expression of pyriproxyfen resistance in *B tabaci* are presented and analysed, and the implications for managing resistance to this insecticide in light of the Israeli experience are discussed.

2 EXPERIMENTAL METHODS

2.1 Insects

2.1.1 Laboratory strains

Insects were reared on cotton seedlings (cv Acala) under standard greenhouse conditions, at 26($\pm$2)°C. A susceptible strain (pyri-S) was used as a reference for evaluating the tolerance of field-collected strains of *B tabaci* to the tested insecticides. This strain was collected from cotton fields during the 1987 season; since then it has been reared in isolation and not exposed to any pesticides.

Another strain (pyri-R), collected from a rose greenhouse in 1992, was found to be highly resistant to pyriproxyfen, and has been maintained on cotton seedlings as described above, but under pyriproxyfen insecticide pressure in the laboratory.[2]

2.1.2 Field collections

From 1991 to 1997, adult whiteflies were collected from commercial cotton fields in two areas in Israel: the Ayalon Valley (Kibbutz Nachshon, in central Israel) and in the western Negev (Kibbutz Kfar Aza, in southern Israel). Collections were made in the early morning, when adults were less active. Leaves containing *B tabaci* adults and pupae were collected, confined in a wooden rearing cage (50 × 35 × 35 cm), and returned to the laboratory. Whiteflies were tested on the day of collection for their susceptibility to pyriproxyfen, as described below.

2.2 Insecticide

Pyriproxyfen was tested as a commercial 100 g litre^{-1} EC ('Tiger'; Sumitomo Co Japan), obtained from the Agan Chemical Company, Ashdod, Israel.

2.3 Bioassay

Cotton seedlings (20–25 cm tall) were dipped in various concentrations of formulated insecticide, or in deionized water (control). Two assays were conducted: a transovarial assay, measuring failure of egg hatch; and an adult emergence assay, measuring pupal mortality.[1] For the former, 20 *B tabaci* females were confined in clip-cages and allowed to oviposit on treated cotton seedlings for 48 h at 26($\pm$2)°C, 60% RH, and under a 14-h photoperiod. Adults were then removed, and egg viability was determined 8–10 days later. In the adult emergence bioassay, plants infested with first-instar *B tabaci* nymphs were dipped in insecticide, and adult emergence and pupal mortality were determined two to three weeks later. Each bioassay was done with at least four concentrations, each with four to 10 replicates.

2.4 Simulator studies

Field simulator studies were conducted at IACR-Rothamsted, UK, using large (170 × 120 × 100 cm) population cages designed to simulate the field effects of insecticides against insect pests and their natural enemies.[7,8] For this experiment, *c*160 adult whiteflies (80 males and 80 females) of the required strain were released onto eight cotton plants within each of four cages. Plants in two of these cages had been sprayed to run-off 3 h previously with pyriproxyfen (40 mg litre^{-1}), corresponding to the recommended application rate. The four regimes investigated were: (1) susceptible strain (pyri-S) sprayed with pyriproxyfen; (2) susceptible strain unsprayed; (3) resistant strain (pyri-R) sprayed with pyriproxyfen; and (4) resistant strain unsprayed. Adult numbers were monitored at *c*3-day intervals for up to 50 days post-spraying, using a rigid borescope (KeyMed, Olympus) inserted through brush borders on the sides of the cage. Cages were maintained at 25($\pm$1)°C under a 12-h photoperiod.

3 RESULTS

Seasonal trends in susceptibility to pyriproxyfen of field populations of *B tabaci* are summarized for the Ayalon Valley and western Negev in Figs 1 and 2 respectively. At both sites, there was (initially) a consistent trend for tolerance to increase within seasons but then decrease by the start of the following season. Until 1995, levels of resistance early in the season in the Ayalon Valley were relatively low, enabling effective control of whiteflies with pyriproxyfen. However, from 1995 onwards, susceptibility of *B tabaci* in this region decreased dramatically and was no longer restored between seasons. There was evidence of a slight reduction of resistance during 1997, due possibly to reduced use of pyriproxyfen on cotton in this area. Furthermore, data for 1998, when pyriproxyfen was used minimally on cotton in the Ayalon Valley and western Negev, show resistance to have declined to levels observed in 1995/6.

In the western Negev, early-season susceptibility to pyriproxyfen did not change substantially until 1997. However, following the use of pyriproxyfen in 1996 and 1997, resistance increased to levels compa-

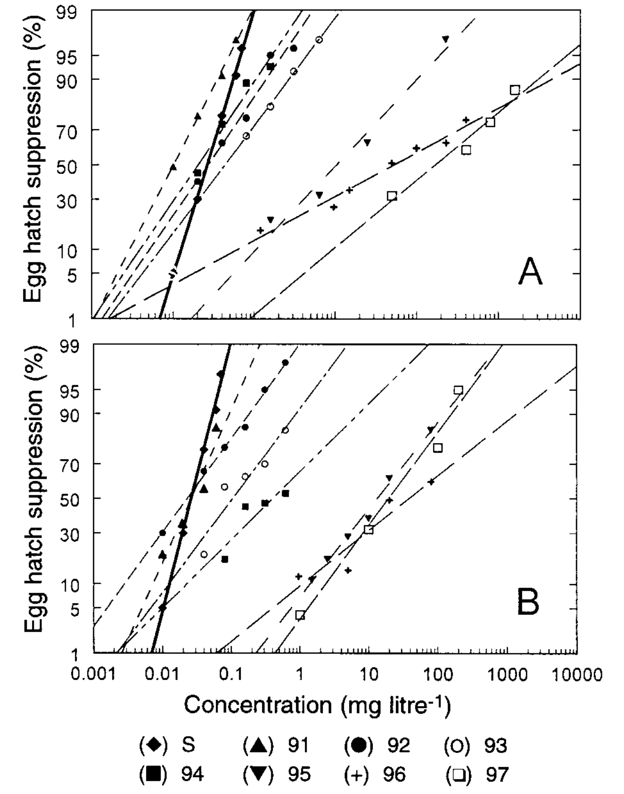

Figure 1. Relationship between ovicidal activity (on a probit scale) and pyriproxyfen concentration for *Bemisia tabaci* populations collected from cotton between 1991 and 1997 from the Ayalon Valley of Israel. A. early season; B. late season.

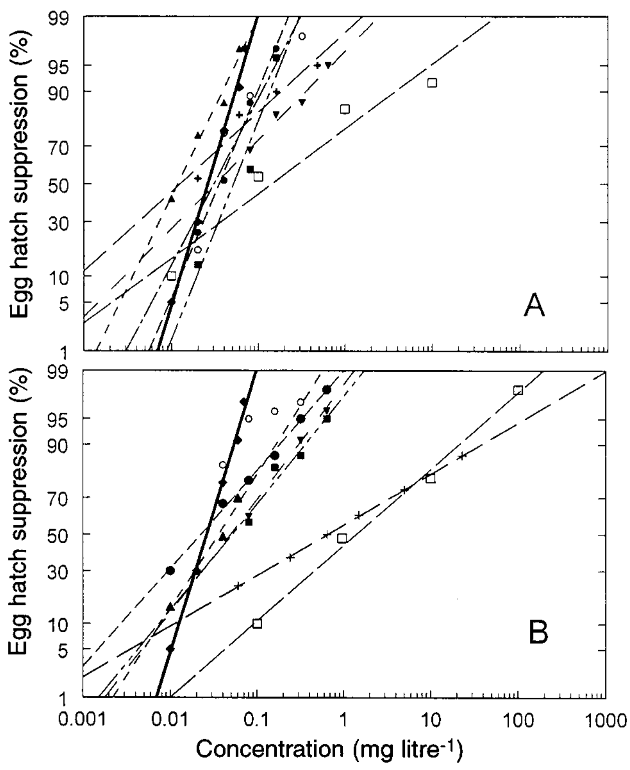

Figure 2. Relationship between ovicidal activity (on a probit scale) and pyriproxyfen concentration for *Bemisia tabaci* populations collected from cotton between 1991 and 1997 from the western Negev in southern Israel. A. early season; B. late season. Symbols as for Fig. 1.

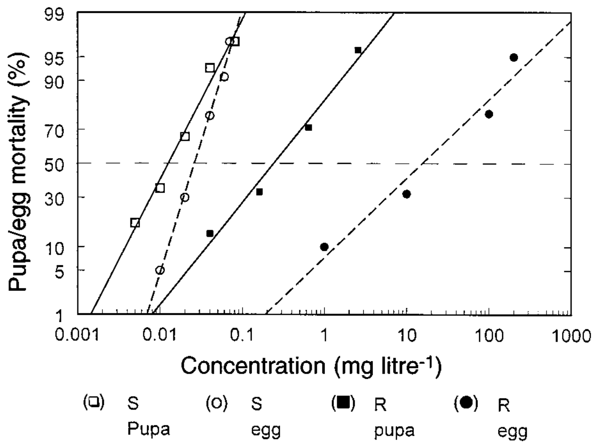

Figure 3. Relationship between pupal and egg mortality (on a probit scale) and pyriproxyfen concentration for *Bemisia tabaci* populations collected from cotton in 1996 from the Ayalon Valley of Israel.

rable to those observed in the Ayalon valley during these seasons (Fig 2). In the Ayalon Valley, resistance was much more strongly expressed in terms of egg hatch than pupal survival (Fig 3). This was consistent with earlier findings for a greenhouse population[6] and this may account for some reports of continued control of *B. tabaci* despite an obvious failure of the ovicidal activity of pyriproxyfen.

Changes in adult population size in field simulators did nonetheless demonstrate a marked reduction in control efficacy by a single application of pyriproxyfen caused by resistance in the pyri-R strain (Fig 4). In the absence of treatment, pyri-S adults built up more rapidly and to greater numbers than those of pyri-R, implying an intrinsic difference between strains in fecundity or development time (or both). Despite this difference between untreated populations, the pyri-S strain was controlled far more effectively than pyri-R, which reached approximately the same population size, after 50 days, irrespective of pyriproxyfen treatment.

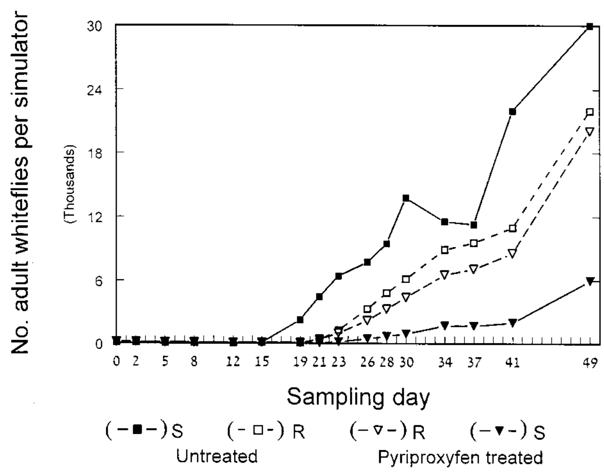

Figure 4. Changes in adult numbers of the pyri-S and pyri-R strains of *Bemisia tabaci* populations treated or untreated with pyriproxyfen (40 mg litre^{-1}) in field simulator cages.

4 DISCUSSION

The history of pyriproxyfen use against *B. tabaci* in Israel provides a striking example of how genetic, ecological and operational factors may combine to promote resistance, despite the existence of a resistance management strategy that limited the use of this compound to a single application per cotton season.[5] This restriction was placed on both IGRs, buprofezin and pyriproxyfen, following their introduction in 1989 and 1991, respectively, as a pre-planned attempt to delay the development of resistance.[3,6] It was facilitated by the outstanding effectiveness of pyriproxyfen, one application of which in many cases proved sufficient to control *B. tabaci* during the whole cotton season. Early observations of an increase in resistance within seasons did not cause alarm, due to the tendency for populations to revert to susceptibility by the start of the following season. The reason(s) for this reversion are still unclear, but could relate to differences in the inherent fitness of pyriproxyfen-susceptible and -resistant genotypes that were apparent from comparing the dynamics of the pyri-S and pyri-R strains in field simulators. In addition, gene flow between treated and untreated hosts could have contributed to diluting the frequency of resistance between successive seasons.

Factors that initially moderated the selection of resistance on cotton have clearly been unable to prevent a gradual increase in the frequency of resistant phenotypes, albeit at different rates in two regions. This increase was first apparent as a flattening of dose-response relationships, which resulted in a poor fit to the probit model, as expected when dealing with genetically heterogeneous populations. However, some recent samples (eg those collected late season from the Ayalon Valley in 1995 and 1997) have yielded steeper and more homogeneous dose-response relationships, and may indicate an approach to fixation of resistance to pyriproxyfen in that area.

Intriguingly, the level of resistance found in the Ayalon Valley after five years of pyriproxyfen use (ie a maximum of five applications) was almost identical to that found following three successive applications of pyriproxyfen in a greenhouse in the western Negev in 1991.[5] Furthermore, resistance in cotton fields located closest to that greenhouse in the western Negev remained relatively low until 1997. Each year in the spring and early summer (April–May), infestations of *B tabaci* occur earlier and develop faster in the Ayalon Valley than in other parts of Israel (unpublished data). The reason for this early infestation is unclear, but, based on resistance monitoring data, it appears that they originate from the same source each year, even though very low numbers of *B tabaci* are considered to overwinter in the Ayalon Valley.[9] Geographically, the Ayalon Valley is relatively isolated, being surrounded by hills that could provide a potent barrier to the movement of *B tabaci*, thereby mimicking the enclosed environment of a greenhouse. Agricultural practices can also affect the evolution of resistance in polyphagous species such as *B tabaci*.[4,5] In the Ayalon Valley, primarily cotton and sunflowers are grown, both of which are excellent host plants of *B tabaci*. Although largely unsprayed, sunflowers are planted and harvested much earlier than cotton, and do not therefore constitute an effective refuge when insecticides are applied against whiteflies on cotton. In the western Negev there is a greater diversity of crops including melons, tomatoes, potatoes, and greenhouse ornamentals, most of which have not been treated recently with pyriproxyfen. The later build-up of whiteflies and diversity of pyriproxyfen-untreated hosts may therefore explain a delayed appearance of resistance in this area. However, these factors have clearly not prevented the development of resistance, which in some areas now threatens to become as common as in the Ayalon Valley. Thus, during the 1998 season, insecticides such as acetamiprid and diafenthiuron were used instead of pyriproxyfen in most cotton fields in Israel. As a result, resistance levels in the Ayalon Valley and western Negev declined to those observed in 1995/6.

Results presented in this paper have potentially important implications for managing resistance to pyriproxyfen in *B tabaci*. It is critical that resistance management strategies are established that take geographic, climatic and agronomic factors into account. In general, a restriction to one application per season appears to be essential for combating resistance to pyriproxyfen. Regions with comparable climates, cropping systems and/or histories of whitefly resistance problems to the Ayalon Valley of Israel that wish to sustain the use of this valuable compound for longer periods may be advised to implement 'pyriproxyfen-free years' in order to promote more effective containment of resistant genotypes.

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